Soil Microsites as a Source of Denitrification Variability[1]

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ABSTRACT

The spatial variability exhibited by soil denitrification rates is high. As is typical for natural denitrification rate measurements, the individual rates of most samples of a given data set are low; however, a few samples often exhibit extremely high rates. Such data are characterized by highly skewed sample frequency distributions. This study was initiated to investigate the underlying mechanisms responsible for these observations. It was found that "hot-spots" of high specific denitrification activity were associated with particulate organic C material in the soil. The high specific activities of these hot-spots (incubated under aerobic conditions with no amendments) were similar to the denitrification activity of the bulk soil measured under conditions of anaerobiosis with added glucose and NO_3. This observation served as the basis of a computer model that evaluates the influence of the density and dispersion pattern of these high activity sites on the measured rates of denitrification. Histograms generated from computer simulations are very similar to histograms obtained for real data, supporting the concept that the patchy dispersion of particulate organic material in soil is a major factor influencing the variability of natural denitrification rates.

Additional Index Words: **lognormal, skewed distributions, particulate C, CO_2 production.**

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SPATIAL VARIABILITY of soil properties has been a topic of high interest, as evidenced by the increased application of novel statistical methods in the analysis of soils data (Nielsen and Bouma, 1985). Due to their dynamic nature, microbial processes and soil properties directly influenced by soil microorganisms typically display high variability and often exhibit skewed frequency distributions that can be approximated by the lognormal distribution family. This recognition has been an important step in dealing with variability in that statistical techniques designed for lognormal distributions can be applied to yield better parameter estimates (Finney, 1941; Aitchinson and Brown, 1957; Sichel, 1966). Whereas consideration of the frequency distribution provides better information about variability than consideration of just point estimates of location and scale (Flühler et al., 1976; Nicot et al., 1984), this approach has not yielded insights into the underlying mechanisms responsible for the variability.

Insight can be gained as to the underlying mechanisms controlling variability by considering the factors that result in skewed distributions. The law of proportionate effects predicts that a variable will exhibit a lognormal distribution when the factors controlling the variable are combined in multiplicative manner (Aitchinson and Brown, 1957). Multiplicative effects have been proposed as the source of the lognormal distributions exhibited by populations of bacteria in the rhizosphere and on leaf surfaces (Loper et al., 1984; Hirano et al., 1982). In a study of community structure, Ugland and Gray (1982) suggest that multiplicative effects yield patchy dispersions of species in nature, which is the ultimate cause for the skewed distributions observed. The concept of a patchy distribution may also apply to the natural denitrification process since, in unsaturated soils, denitrification is presumed to occur in anaerobic microsites (Dowdell and Smith, 1974; Flühler et al., 1976; Tiedje et al., 1984).

The highly skewed frequency distributions of de-

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nitrification indicate that most samples of a given data set exhibit low rates but a few samples have very high rates. It is the high rates of these few samples that result in the high observed variability. This study was undertaken to determine the underlying mechanisms controlling the variability of denitrification in soil.

MATERIALS AND METHODS

Field Site and Sampling.

Samples were collected from a field site located on the Univ. of Maryland Plant Res. Farm, Beltsville, MD. The site had established plots of no-till continuous corn (12 yr). The soil at this site was a Beltsville silt loam (Typic Fragiudult) having a pH of 6.5 and total organic N (Kjeldahl) and C (persulfate digestion) contents of 0.8 and 5.1 g kg^{-1}, respectively. Soil cores were obtained by pounding steel tubes, which were fit with hardened cutting bits, into the ground to a depth of 16 cm. The intact soil cores contained within the steel tubes were then slid into stoppered plastic tubes. The soil cores fit loosely in the plastic tubes to facilitate gas diffusion into and out of the soil. Upon returning to the laboratory, denitrification rates were measured on the intact soil cores. Soil samples were collected with five different sized soil cores ranging from 1.73 to 5.4 cm in diameter as part of a larger study to investigate the influence of sample size on denitrification (Parkin et al., 1987). Rate data of all but the smallest core size were analyzed as a single data set since they were not significantly different as determined with the Kolmogorov-Smirnov test and Tukey's test on both untransformed and log-transformed rate data ($p < 0.05$).

Natural Denitrification Rate Measurements

Denitrification rate measurements were begun immediately upon returning to the laboratory. Natural denitrification rates of the intact soil cores were estimated using an C_2H_2 block technique. First, the gas pressure in the cores was brought to atmospheric levels by venting the cores with a needle. After the cores were vented, the appropriate volume of C_2H_2 was added to each core to achieve a final C_2H_2 partial pressure of approximately 10 kPa in each of the different sizes of soil cores. All C_2H_2 gas used for the denitrification incubations was generated by reacting CaC_2 with distilled water immediately prior to use. The pressure increase resulting from the C_2H_2 addition was then observed in each core using a pressure transducer equipped with a 55.2 kPa (8 psi) bellows (Unimeasure, Inc., Grants Pass, OR)[3]. The pressure readings were used to calculate the total gas filled volume in each of the samples as described by Parkin et al. (1984).

Gas in the soil cores was mixed to distribute C_2H_2 throughout the soil pores. Mixing was accomplished by alternately drawing and releasing a vacuum on the samples using a 60-cm^3 syringe. A device was constructed to facilitate this procedure, whereby four soil cores could be mixed at once (Parkin et al., 1987). The mixing procedure and the loose fit of the intact soil cores in the tubes facilitated both C_2H_2 distribution into and N_2O distribution out of the soil pores.

Following the gas mixing, the overpressure of gas in the soil cores resulting from the initial C_2H_2 injection was vented. Cores were incubated at 24 to 26°C and gas samples withdrawn at 3-, 6-, and 18-h time points following the C_2H_2 mixing. Gas samples were obtained by adding 5 mL of air to each core, mixing the gas in the cores, then removing a 5-mL gas sample. The 5-mL gas samples were stored in 3-mL evacuated vials (Beckton Dickinson Co., Rutherford, NJ) for later N_2O analyses.

Contents of the vials were analyzed for N_2O using an electron capture detector-gas chromatograph (Tracor model 222 or Shimadzu mini-2) equipped with an automatic gas sampler (Parkin, 1985). Denitrification rates were then calculated from the rates of N_2O production after correcting for the dilution which resulted in the 5-mL air additions to the cores at each time point and correcting for the N_2O dissolved in the soil water, which was determined gravimetrically. Rates are expressed on a gram dry weight soil basis.

Denitrifying Enzyme Activity

Immediately following the last gas sampling point of the intact core incubations, denitrification enzyme activity was measured (Smith and Tiedje, 1979; Tiedje, 1982). The soil samples were sieved, mixed, and a 25-g subsample placed into a 125-mL Erylenmyer flask containing 25 mL of a solution containing 1 m*M* glucose, 1 m*M* KNO_3, and 1 g/L of chloramphenicol. The soil slurries were made anaerobic by alternately flushing with Ar and evacuating the flasks four times. Five-milliliter gas samples were withdrawn 0.5, 1, 1.5, and 2 h following the addition of 20 mL of C_2H_2 to the flasks. Gas samples were stored in evacuated vials and analyzed for N_2O as described above.

Core Segmentation Experiments

Experiments were conducted to identify specific sites of denitrification activity in soil. First, the denitrification rate of an intact soil core (15 cm long) was determined over a 18-h period. Following this initial rate determination, the core was sectioned into three 5-cm segments and the denitrification rates of these segments determined over a 12-h period. The 5-cm segment that exhibited the highest activity was sectioned into five 1-cm segments, which were incubated separately (12-h incubation). Finally, the 1-cm sections that had the highest rates (usually the 0–1 or the 1–2 cm segments) were dissected and the particulate organic material was incubated separately from the inorganic soil material (7-h incubation). All incubations were done aerobically in the presence of approximately 10 kPa C_2H_2 (resulting O_2 level of ca. 18 kPa). This tedious protocol, which was repeated on 12 different soil samples, allowed the determination of the specific denitrification rates of the particulate organic fractions and the inorganic fractions present in soil.

Descriptive Model of Denitrification

A model was developed to describe the influence of the heterogeneous dispersion of denitrification activity in soil on the measured denitrification rate. This model is based on the assumption that discrete microsites of denitrification exist in the soil and that an aggregated dispersion of these microsites results in localized zones of very high denitrification activity.

The model is, in essence, a computerized representation of the soil in which an artificial field is constructed, and within this "field" random locations (points) are designated. These points represent sites of in situ denitrification activity in the soil. Localized zones of high denitrification activity are represented by clusters or patches of these points. The clusters are generated using an algorithm described by Green (1979) which generates an aggregated spatial distribution of points. This algorithm randomly selects the coordinates of a seed point. Additional random points are then selected. These additional points are rejected or accepted depending on the following criteria: (i) if point is less than a user specified distance (D) from the seed point it is accepted, (ii) if the point is greater than D from the seed point it is rejected, and (iii) if the point is separated by exactly D from the seed point it has a 50% probability of being rejected.

[3] Mention of a product by company or name is not an expressed or implied endorsement by the USDA.

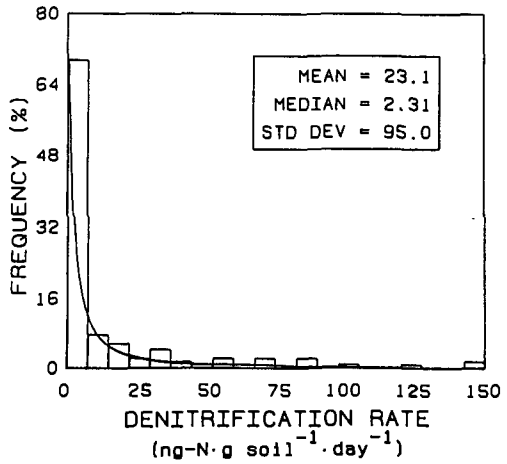

Fig. 1. Histogram of 144 denitrification rates as determined in intact soil cores. The solid curve is the lognormal probability density model calculated from the statistical parameters of the data.

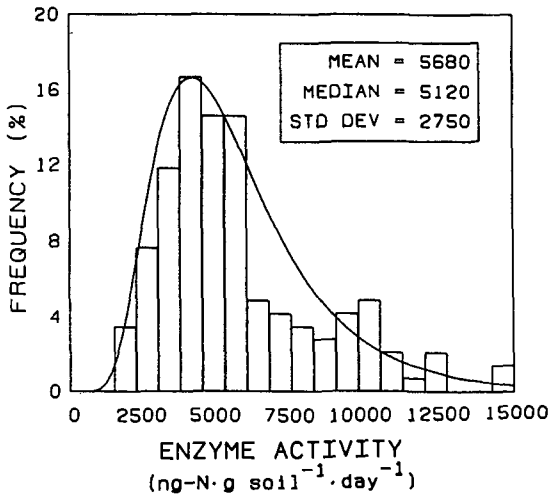

Fig. 2. Histogram of 144 maximum potential denitrification measurements. The solid curve is the lognormal probability density model calculated from the statistical parameters of the data.

After the field is constructed, a denitrification rate is assigned to each point. The denitrification rate values are randomly selected from a user defined probability density function. The resulting artificial field is analogous to the natural soil situation in which there are locations where denitrification activity is expressed (anaerobic microsites) and locations where activity is zero (aerobic sites, sand grains, and pebbles).

The field is randomly "sampled" after designating the sample size and sample number. Each sample contains a random number of both denitrifying sites as well as zones of zero activity. The resulting rate of each individual sample is then calculated by averaging the values of the high activity locations over the entire area contained by the sample. It must be realized that, since the input parameters are empirically assigned, this model has no predictive power and can only function in a descriptive sense to evaluate the interactions between the patchy spatial dispersions and sample size on variability.

RESULTS

Estimates of natural denitrification rates measured with intact soil cores were highly variable and displayed a skewed frequency distribution (Fig. 1). This distribution was approximated by a lognormal probability density function. It is apparent that while most of the rates were low, some samples exhibited extremely high rates. The median rate was 2.36 ng-N g^{-1} d^{-1}, which was one order of magnitude less than the mean rate (23.1 ng-N g^{-1} d^{-1}). The high variability is apparent by observing the magnitude of the standard deviation.

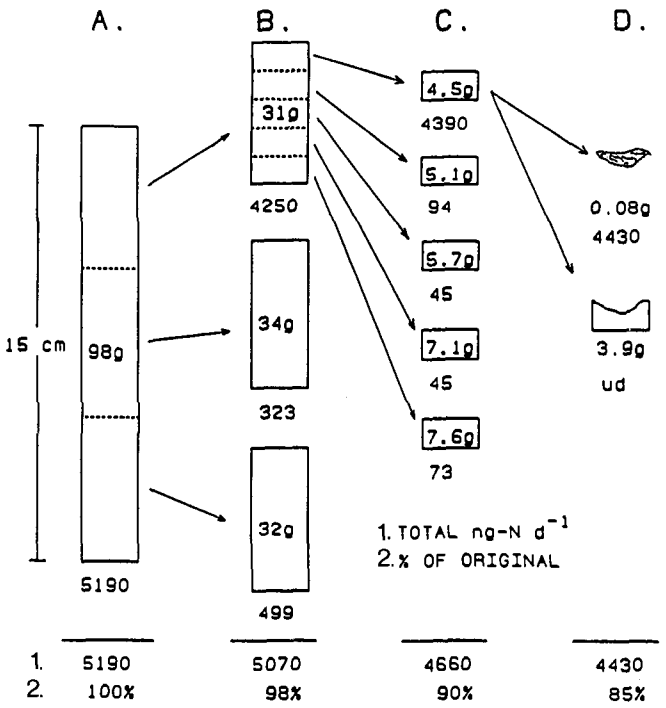

Fig. 3. Results of a core segmentation experiment to identify active denitrifying sites in soil. All incubations were conducted under a headspace atmosphere of 18-kPa O_2 and 10-kPa C_2H_2.

Denitrification enzyme activity was also skewed and approximated a lognormal distribution (Fig. 2). Since these incubations were conducted under conditions optimized for denitrification (anaerobic + glucose + NO_3^-), these measurements indicate the maximum denitrification potential of the soil and, therefore, are much higher than the rates of the undisturbed soil core. A mean rate of 5680 ng-N g^{-1} d^{-1} was determined. The relative variability of these incubations is substantially less than the intact core incubations. Since all the major factors that control denitrification have been optimized, the variability associated with these measurements is the variability due only to the dispersion of potentially active denitrifying enzymes in soil.

With regard to the variability of the intact core rates, it is apparent from Fig. 1 that a significant portion of the variability is due to the occurrence of occasional samples having high rates. It was hypothesized that the high rates were due to the nonhomogenous dispersion of active denitrification microsites in the soil. This hypothesis was tested by resampling the field and performing a series of incubations to isolate and identify the source(s) of high denitrification activity within a given sample. Figure 3 shows the results of one such incubation.

Table 1. Specific rates of dentrification and CO_2 production for soil particulate organic material and inorganic soil material.†

Material‡	Weight	Dentrification	CO_2 production
	g	ng N_2O-N g^{-1} d^{-1}	μg CO_2-C g^{-1} d^{-1}
Beetle carapace	0.008	2 520	45 000
Plant root	0.068	510	1 950
Plant root	0.137	21 400	3 640
Pigweed leaf	0.080	55 400	3 640
Plant root	0.398	8 100	6 780
Soil	5.6	12.7	198
Soil	3.6	und§	98
Soil	7.6	0.5	36
Soil	5.1	18.4	und
Soil	7.1	6.3	und
Soil	7.7	14.3	und
Soil	9.3	4.8	und

† All incubations were conducted under aerobic conditions (ca. 18-kPa O_2).
‡ Particulate organic fractions were picked clean of all visible aggregated soil material and the particulate organic material was removed from the soil fraction.
§ und = rates were undetectable.

The initial rate of an intact 15-cm, 98-g soil core was 5190 ng-N d^{-1} (Fig. 3A). Most of the activity was associated with the top 5-cm section of the soil core, which had a rate of 4390 ng-N d^{-1} (Fig. 3B). This section was further divided and it was found that the top 1-cm piece (4.5 g) had the highest activity (Fig. 3C). In this 1-cm section a folded piece of decaying pigweed leaf (*Amaranthus* spp.) was identified. This material was carefully unfolded and separated from the inorganic soil material. Aerobic incubations (18-kPa O_2) of these two fractions revealed that the particulate organic fraction was responsible for all the activity observed in the top 1-cm section of the soil core and the denitrification was not detectable in the inorganic soil fraction (Fig. 3D). The resulting activity of this "hot-spot" of denitrification (80 mg of particulate organic material) accounted for 85% of the total activity of the intact 98-g soil core.

These segmentation-incubation experiments were performed on 12 different soil cores; however, denitrification hot-spots were not detected in every sample. Five denitrification hot-spots were isolated from four of the soil cores. In the remaining eight soil cores no hot-spots were detected and denitrification activity was observed to decrease with each fractionation step, indicating that, in these samples, soil structure was important in maintaining anaerobic conditions conductive for denitrification. In the four cores that exhibited these stable hot-spots, from 25 to 85% of the total denitrification activities of the intact soil cores were associated with the particulate organic material, and this particulate material represented from 0.4 to 0.08% of the total masses of the soil cores.

The specific denitrification rates of the five isolated hot-spots were several orders of magnitude greater than the denitrification rates of inorganic soil material (Table 1). The particulate C in these incubations was separated from any visible aggregated soil structures, and, since these incubations were performed under a headspace of 18 kPa O_2, it appears that anaerobic conditions necessary to support the high denitrification rates were maintained by high rates of O_2 consumption. This is supported by the high specific rates of CO_2 production also observed by the particulate C material. Rates of CO_2 production of the particulate C material were several orders of magnitude greater than rates of CO_2 production from the soil.

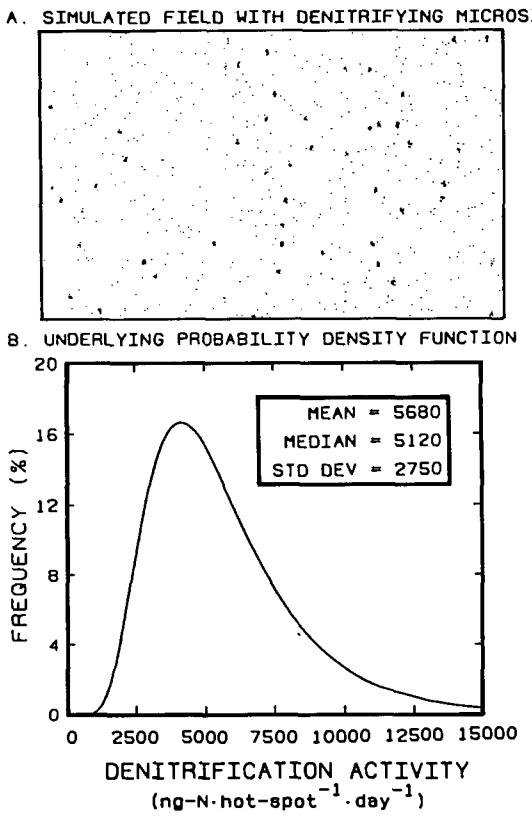

Fig. 4. Descriptive model of soil denitrification. (A) Hot-spots (points) are scattered throughout a "field" and, (B) denitrification activity values are assigned to these hot-spots from an underlying population distribution similar to that exhibited by the denitrifying enzyme activity.

From the great difference in the denitrification rates associated with the particulate C as compared to the inorganic soil, it is assumed that the presence or absence of a high denitrifying piece of particulate organic C in a given soil sample would have a significant impact on the measured denitrification rate of the sample. It was on this basis that a preliminary model was developed to describe the influence of denitrifying hot-spots on the variability of denitrification rate measurements.

To implement this model an artificial field was created in which denitrifying hot-spots (points) were dispersed (Fig. 4A). These points are the locations where denitrification activity is being expressed in situ. In Fig. 4A, 1000 dentrifying points were dispersed over a possible 240 000 locations in the field. Of these 1000 denitrifying points, 500 points were dispersed randomly over the field in a uniform dispersal pattern and 500 points were dispersed in an aggregated pattern as 50 patches or clusters, which contained an average of 10 points each.

Rates were then assigned to each of the designated denitrifying points from the probability density function shown in Fig. 4B. The probability density function used for these simulations was a lognormal dis-

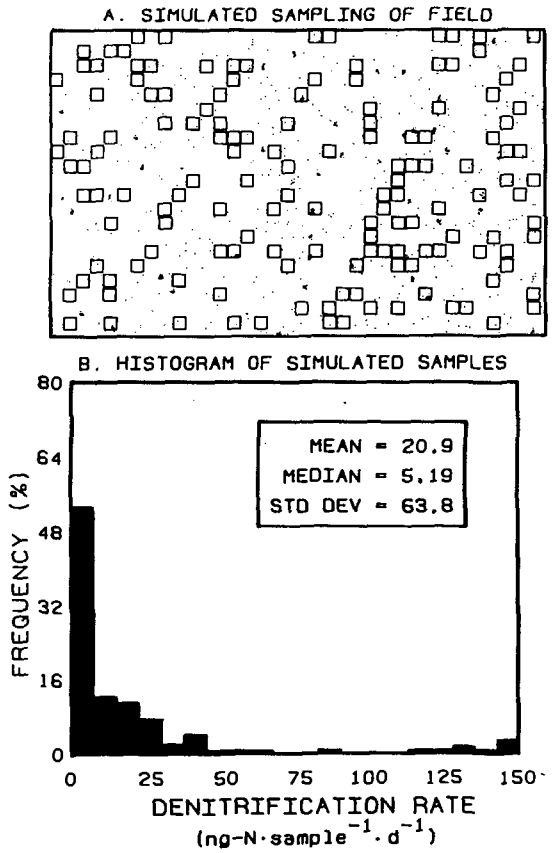

Fig. 5. (A) The artificial field is sampled (squares) and the denitrification rate of each sample is calculated. (B) Histogram of computer generated samples values and statistical parameters.

tribution that corresponded to the distribution observed for the measured denitrification enzyme activity rates shown in Fig. 2. Since the actual form of the distribution function associated with denitrifying microsites in soil was unknown, this seems a reasonable approximation, as the denitrification rates of the particulate organic material (Table 1) were of the same magnitude as rates of denitrification enzyme activities in soil. Also, at sites in the soil where denitrification is expressed in situ, anaerobic conditions as well as C and NO_3^- presumably exist. These were the conditions under which the denitrification enzyme activity incubations were performed.

The field was sampled (Fig. 5A) by collecting 144 randomly located samples (squares). Each sample confined 240 locations; thus, for this particular simulation run, the microsite density was 1 microsite per sample on the average. However, due to the patchy dispersion, some samples contained several denitrifying sites and some samples contained no denitrifying points. The denitrification rate of each sample was calculated by averaging the rates associated with the hot-spots contained in each sample over the entire area encompassed by the sample. This is analogous to the denitrification rate measurements in the intact core samples. The measured rate of an intact soil core is the average of all the hot-spots (denitrifying microsites) and cold-spots (aerobic sites, sand grains, pebbles, etc.) within the sample. The resulting histogram of the simulated denitrification rates (Fig. 5B) exhibits a much higher degree of skewness than the underlying population distribution (Fig. 4B) and is very similar to the actual data (Fig. 1) with respect to both the shape as well as the magnitudes of the mean, median, and standard deviation.

DISCUSSION

Microbial denitrification is an anaerobic process. A popular hypothesis often invoked to explain the occurrence of denitrification in apparently well-drained soils is the existence of anaerobic microsites within soil aggregates (Dowdell and Smith, 1974; Flühler et al. 1976; Tiedje et al., 1984). This concept has been based on the work of Currie (1961) and Greenwood (1961), who describe the factors influencing the aeration state of soil aggregates. Direct measurements of O_2 concentrations in soil aggregates have corroborated the postulation of anaerobic zones in soil aggregates (Greenwood and Goodman, 1967; Sexstone et al., 1985), and mathematical models of soil denitrification that incorporate this concept have been developed (Leffelaar, 1979; Smith, 1980). However, if O_2 consumption rates are great enough, the limitation imposed on O_2 diffusion by aggregate structure is not necessarily a prerequisite for the development of anaerobic conditions.

In this study particulate organic material supported high specific rates of denitrification in the absence of visible aggregated soil material. Since these incubations were conducted under an atmosphere of 18 kPa of O_2, presumably anaerobic conditions resulted from high O_2 consumption rates, thus allowing denitrification to occur. The specific denitrification activities reported for the particulate organic material were one to two orders of magnitude higher than the specific rates of individual soil aggregates supporting anaerobic zones reported by Sexstone et al. (1985).

The high CO_2 production rates of the particulate organic C indicate a high O_2 consumption potential. If O_2 consumption rates are great enough, anaerobiosis can develop, even if only a thin film of water is impeding O_2 diffusion. It was calculated, for the pigweed leaf in Fig. 3., that given an O_2 diffusion coefficient in water of 10^{-5} cm^2 s^{-1} and a CO_2 production (O_2 consumption) rate of 3.38×10^{-6} cm^3 cm^{-2} s^{-1}, a water (or microbial) film of at least 160-μm thick must cover the leaf in order to achieve anaerobic conditions at the leaf surface. This assumes that CO_2 production was uniform over the entire leaf surface. If O_2 consumption was occurring in localized zones on the leaf surface, then a much thinner water or bacterial film would be necessary to enable anaerobic conditions. For example, if all the CO_2 production observed in Fig. 3D was occurring on 10% of the leaf surface, then a water film (or microbial film) of only 16 μm is necessary to achieve anaerobic conditions. These calculations are supported by the calculations of Strand and McDonnell (1985), which predict that biofilms 19 μm thick can support denitrifying conditions.

The observation of high specific rates of denitrification associated with particulate C suggest that the patchy distribution of particulate organic material is a significant factor influencing the magnitude and variability of natural denitrification rates in soil. The high variability associated with estimates of natural soil denitrification rates (Rice and Smith, 1982; Folorunso

and Rolston, 1984; Parkin et al., 1985) may be a direct result of patchy distributions of denitrification activity in soil. Spatial analysis of variability indicates that denitrification exhibits a high degree of small scale variability (nugget variance), suggesting that spatial discontinuity at the small scale is a major component of the total observed variability (Folorunso and Rolston, 1984; Parkin et al., 1987).

A descriptive model was developed to integrate the observation of patchy dispersion of denitrifying sites in soil with the highly skewed distributions exhibited by natural denitrification rate estimates. The underlying assumption of this model is that hot-spots of denitrification are nonhomogenously dispersed in soil. Therefore, a single soil sample represents a bulked average of the high and the zero activity sites within that sample. The sample frequency distributions resulting from this model are highly skewed and approximate the lognormal distributions displayed by the actual field data.

The denitrification rates obtained from this model are a function of both the shape of the underlying probability density function of the rates associated with the hot-spots as well as the dispersion pattern and density of the microsites in relation to the sample size selected. It is likely that, in nature, the dispersion of hot-spots, and possibly the probability density function of hot-spots vary temporally in response to changing conditions in the soil (e.g., moisture). However, at this time, insufficient data exists concerning dispersion of hot-spots and the magnitude of the denitrification rates associated with hot-spots. Thus, until additional data becomes available, this model can function only in a descriptive sense. However, the proposed model does illustrate that highly skewed sample distributions can result from the patchy spatial distribution of the underlying variable.

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